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Original scientific paper

AN ASSESSMENT OF NUTRITIONAL QUALITY OF HYBRYD MAIZE GRAIN BASED ON CHEMICAL COMPOSITION

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The aim of this study was to investigate chemical composition of grain of 20 maize hybrids, from different maturity groups, to define their nutritional quality from the point of main grain constituents: starch, total proteins, soluble proteins, oil, phytate, inorganic phosphorus, and soluble phenolics, as well as mass of 1.000 grains. A set of 20 ZP hybrids, FAO 400-800, were grown in a randomized complete block design (RCBD) at Zemun Polje (Serbia), during the summer of 2009. Average content of proteins, oil and starch of analyzed maize hybrids was 10.5%, 4.7% and

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80.8%, respectively. Hybrids from FAO maturity group 600 had highest grain mass (in average 419.5 g). Genetic variability in seed phytate content was observed, with values ranging from 2.64 to 3.75, averaged 3.44 g kg^{-1}. Inorganic phosphorus (Pi) concentrations were between 0.120 µg g^{-1} (ZP 805) to 0.415 µg g^{-1} (ZP 588ex), averaged 0.24 µg g^{-1}. Content of soluble proteins varied from 54.33 to 78.74 mg g^{-1}. The high positive correlation was observed between total proteins and oil content in hybrids, as well as between starch and phenolics content. Phytate content was positively correlated with proteins, but this correlation was not significant, while the correlation between phytate and mass of 1,000 grains was positive and significant. A negative correlation occurred between phytate and starch.

Key words: grain content, maize, nutritional value

INTRODUCTION

Grain quality is a wide term that means different things to corn producers, crop consultants, dairy producers or ruminant and human nutritionist. From the nutritional point of view corn grain such as fat, protein, hardness, density, and starch content define corn quality characteristics. Chemical content of maize grain is very important for human and animal diets. Average content of proteins, oil and starch in maize seed is 9%, 4%, 73%, respectively (BALCONI *et al.*, 2007). Some breeding programs are routing the specific traits for different usage of maize grain, such as increasing of oil, protein or starch content in grain (SALEEM *et al.*, 2008; ROSULJ *et al.*, 2002, POLLAK and SCOTT, 2005, HARRELSON *et al.*, 2008, IDIKUT *et al.*, 2009).

The interaction between different components of maize grain could induce different digestibility of each compound, what could also define nutritional quality of maize grain. HOFFMAN and SHAVER (2011) defined basic properties of nutritional quality of maize grain as feedstuff. Dry grain of flint and dent genotypes contains more hydrophobic prolamin-zein as compared to floury or opaque corns. The crude protein, prolamin protein is negatively related to starch degradability. The cultivars with higher content of crude protein had higher digestibility coefficient of these nutrients, while the nitrogen balance and retention were not influenced by cultivar (JAN *et al.*, 2008). ZILIC *et al.* (2011) ascertained that the highest nutritive value was recorded in sweet maize hybrids, but the low starch and lignin content affected dry matter digestibility.

Most of the phosphorus in maize grain is not nutritionally available to animals and humans, because it is stored in phytic acid that cannot be digested by monogastric animals because they lack the enzyme phytase needed to utilize this *P* form. Nearly all the phytic acid found in mature seeds is bound to minerals in form a mixed cation salt – phytate (LOTT *et al.*, 2000) and could also form protein-phytate insoluble complexes, considering it as a limiting factor to protein utilization. From that point of view, *P* bioavailability in maize diets is only 15% for monogastric animals. Most of the endogenous *P* in the diets of swine and poultry passes into the feces and onto the landscape, where it contributes to surface water pollution. Such contamination can cause eutrophication in aquatic ecosystems, promoting algalblooms and reducing available oxygen for macro-organisms (ERTL *et al.*, 1998). On average, swine need 2.35 g P_i kg^{-1} dietary dry matter to meet their nutritional requirements (KNOWLTON *et al.*, 2004), and phytate levels should be as low as possible to minimize *P* pollution. Therefore, it would be desirable to increase the concentration of inorganic *P* as an available *P* form, and reduce the phytate concentration in maize grain to help alleviate the associated nutritional and environmental problems simultaneously. Low phytate maize is receiving considerable attention as a means of minimizing manure-*P*, with increasing availability of *P* and *Ca* (CAST, 2002; RABOY *et al.*, 1997). Animal feeding trials have shown that *P* availability is greatly improved in low phytate maize genotypes leading to substantial decreases in feces *P* concentration (KNOWLTON *et al.*, 2004; HILL *et al.*, 2009; BOHLKE *et al.*, 2005).

Bound phenolics in grains are associated with cell wall fiber or protein and escaped gastric and small intestine digestion. Soluble phenolics could form indigestible protein-phenolics complexes, what could limit protein utilization. Meanwhile, soluble phenolics, which could be potentially absorbed in small intestine and contributes to the antioxidant activity of body tissues (KLJAK *et al.*, 2009).

The aim of this study was to investigate chemical composition of grain of 20 maize hybrids, from different maturity groups, to define their nutritional quality from the point of main grain constituents: starch, total proteins, soluble proteins, oil, phytate, inorganic phosphorus, and soluble phenolics, as well as mass of 1,000 grains.

MATERIALS AND METHODS

A set of 20 ZP hybrids, FAO 400–800, were grown in a randomized complete block design (RCBD) at Zemun Polje (Serbia), during the summer of 2009. The hybrids were allowed to open pollinate, and two rows were hand-harvested to collect grain samples. Total protein, oil, starch, phytate, P_i, phenolics and soluble protein content were measured. Seed protein, oil and starch content were determined by near-infrared reflectance spectroscopy (NIRS) using Infratec 1241 Grain analyzer, (Foss Tecator, Sweden) and expressed in a percentage of absolutely dry matter of grain (ADM). Seeds for phytate, P_i, phenolics and soluble proteins were grinded in a TecatorKnifetec 1095 sample mill (particle size 0.5 mm) and then the extraction was performed with double distilled water (*DD* H_2O). Phytate was determined colorimetrically by the method of LATTA and ESKIN (1980), modified by SREDOJEVIC and DRAGICEVIC (2009), based on the pink color of the Wade reagent, which is form upon the reaction of ferric ion and sulfosalicilic acid, and has an absorbance maximum at λ=500 nm. In the presence of phytate, the iron is sequestered and unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity. Inorganic P (P_i) was determined according to POLLMAN (1991) with ammonium heptamolybdate + ammonium metavanadate reagent, while the content of soluble proteins was determined by LOWRY *et al.* (1951), using the the Folin-Phenol reagent. Content of total soluble phenolics were determined by the method of SIMIC *et al.* (2004, 2005) with Prussian blue assay. Mass of 1.000 grains was determined gravimetrically.

The experimental data were statistically processed by analysis of the variance (ANOVA) in program package MSTAT-C and analyzed by the LSD-test (5%). Correlations for mean values of chemical components maize grain were determined in program package STATISTICA 10 by using option Spearman Rank Order Correlations. Relations between different grain constituents were presented graphically, using MINITAB 14 software.

RESULTS AND DISCUSSION

Grain chemical composition is the most important trait to the end-use quality of maize. Analysis of variance (ANOVA) showed that all analyzed characteristics were significantly different at level p = 0.05. It is well known that total protein composition varies among genotypes (LORENZ *et al.*, 2007; MLADENOVIC DRINIC *et al.*, 2009). The total protein of 20 ZP hybrids ranged from 8.0% in ZP 805 to 14.0% in ZP 588ex, with average value of 10.5% (Table 1).

The highest protein content has hybrids from FAO 500, while the protein content is significantly decreasing with increase of maturity group. The oil content ranged from value of 4.1% (ZP 653ex) to 5.4% (ZP 666), with average value of 4.7%. The highest average oil content has hybrids from FAO 400, as well as ZP 666 and ZP 708ex. According to fact that starch is main constituent of maize grain (BALCONI *et al.*, 2007), it content ranged from 77.4% (ZP 588ex) to 84.8% (ZP 768), with average content of 80.8%. ZP hybrids belonging to FAO 800 have the highest starch content, but the lowest content of total protein, indicating different endosperm constitution (GOOSSENS *et al.*, 1988). Obtained values for protein, oil and starch are typically for maize genotypes that were not specifically selected for those traits (POLLAK and SCOTT, 2005; HARRELSON *et al.*, 2008; IDIKUT *et al.*, 2009). The mass of 1.000 grains varied upon hybrids from 284.7 g (ZP 753 ex) to 467.7 g (ZP 606). FAO maturity group 600 is characterized with highest grain mass (in average 419.5 g). The highest grain mass in hybrids that belongs to same FAO maturity group was also reported by STEVANOVIC *et al* (2012).

Substantial quantitative variation in phytate has been observed among ZP hybrids (Table 2). The phytate content varied from 2.64 µg g^{-1} to 3.75 µg g^{-1}, averaged 3.44 µg g^{-1}. In general, hybrids from FAO 800 maturity group had the lowest phytate content (in average 2.89 gkg^{-1}), what could serve as a low-phytic feed in nutrition and processing (BOHLKE *et al.,* 2005). Inorganic phosphorus (P_i) is important form of phosphorus in grains, although it is present at relatively low concentrations and thus constitutes a small fraction of the total P of grains. High level of inorganic P is regarded as desirable form a nutritional standpoint. Among hybrids P_i content ranged from 0.120 μgg^{-1} (ZP 805) to 0.415 μgg^{-1} (ZP 588ex), averaged 0.24 µg g^{-1}. The highest P_i content was obtained in ZP 588 ex (0.415μgg^{-1}). Both phytate and P_i values were within the range of values reported for maize hybrids in previous study (RABOY *et al.*, 2000), but hybrids with lower phytate content did not match higher P_i contents, as desirable P form. Content of soluble proteins ranged from 54.33 mg g^{-1} in hybrid ZP 846ex to 78.74 in hybrid ZP 341, with average value of 64.45 mg g^{-1} (Table 2). The observed values mark up that content of soluble proteins in total protein content could vary in wide range: from 52%, present in hybrids ZP 588ex and ZP 666, up to 86% in ZP 805. Such situation could indicate different digestibility and potential of grain usage (BARTECZKO *et al.*, 2008; ZILIC *et al.*, 2011). Soluble phenolics have value from 421.5 μg g^{-1} in hybrid ZP 555 to 557.3 μg g^{-1} in ZP 805 with average value of 485.35 μg g^{-1}.

Table 1. The content of Total proteins, Oil, Starch and Mass of 1000 grains of 20 ZP hybrids (FAO 400-800) and ANOVA for analyzed characteristics

Hybrid	Total Proteins %	Oil %	Starch %	Mass of 1000 grains g
ZP 341	10.05 HI	4.30 FG	79.05 EFG	418.0 C
ZP 434	11.70 BCD	4.90 CDE	79.40 EFG	324.7 O
ZP 444 ex	10.30 GHI	5.15 ABC	78.55 FG	410.4 E
ZP 484 ex	10.35 FGHI	5.00 BCD	81.65 BC	360.4 K
ZP 555	11.15 CDEF	4.50 EF	80.15 DE	377.7 H
ZP 560	11.75 BC	4.95 BCD	78.40 GH	412.0 D
ZP 588 ex	14.00 A	5.05 ABC	77.40 H	398.7 F
ZP 600 ex	9.90 HIJ	4.40 FG	81.55 BC	454.0 B
ZP 606	9.75 IJ	4.30 FG	80.70 CD	467.7 A
ZP 653 ex	10.65 EFGH	4.05 G	81.75 BC	363.7 J
ZP 666	11.40 BCDE	5.45 A	79.65 DEF	392.8 G
ZP 708 ex	12.05 B	5.35 AB	78.55 FG	303.3 Q
ZP 728 ex	11.00 CDEFG	4.60 DEF	80.65 CD	365.2 J
ZP 753 ex	9.65 IJ	4.35 FG	82.20 B	284.7 R
ZP 768	8.50 KL	4.25 FG	84.75 A	367.6 I
ZP 801 ex	10.60 EFGH	5.30 ABC	82.30 B	328.7 N
ZP 805	8.00 L	4.50 EF	82.55 B	325.0 O
ZP 808	8.35 KL	4.25 FG	82.35 B	313.1 P
ZP 836	10.90 DEFG	5.05 ABC	81.50 BC	347.3 L
ZP 846 ex	9.10 JK	4.25 FG	82.40 B	344.7 M
Average	10.5	4.7	80.8	349.43
LSD 0.05	0.83	0.40	1.12	1.54

A-L Means followed by the same letter within the same columns are not significantly different ($p < 0.05$)

Table 2. The content of P_i, Phytate, Soluble Proteins and Phenolics of 20 ZP hybrids (FAO 400-800) and ANOVA for analyzed characteristics

Hybrid	Phytate μg g^{-1}	P_i μg g^{-1}	Sol. Proteins mg g^{-1}	Phenolics μg g^{-1}
ZP 341	3.580 ^{ABC}	0.210 ^{EFGHI}	78.74 A	546.5 ^{AB}
ZP 434	3.300 ^{CDEF}	0.260 ^{CDE}	60.99 FG	478.0 ^{DEFGH}
ZP 444 ex	3.190 ^{DEFG}	0.300 ^{BCD}	69.49 BC	432.9 ^{GHI}
ZP 484 ex	3.120 ^{FG}	0.235 ^{DEFGH}	68.49 C	472.2 ^{DEFGH}
ZP 555	3.390 ^{CDEF}	0.210 ^{EFGHI}	68.76 C	421.5 ^{HI}
ZP 560	3.540 ^{ABC}	0.190 ^{FGHI}	66.72 CD	444.4 ^{FGHI}
ZP 588 ex	3.405 ^{CDE}	0.415 ^{A}	72.62 B	479.4 ^{DEFGH}
ZP 600 ex	3.180 ^{DEFG}	0.175 ^{HIJ}	68.66 C	471.5 ^{DEFGH}
ZP 606	3.450 ^{BCD}	0.255 ^{CDEF}	59.01 GH	447.2 ^{EFGHI}
ZP 653 ex	3.710 ^{AB}	0.220 ^{EFGHI}	64.80 DE	503.7 ^{ABCDE}
ZP 666	3.335 ^{CDEF}	0.250 ^{CDEF}	59.29 GH	408.7 ^{I}
ZP 708 ex	3.445 ^{BCD}	0.350 ^{AB}	56.69 HI	519.4 ^{ABCD}
ZP 728 ex	3.140 ^{EFG}	0.225 ^{EFGH}	59.90 FGH	452.2 ^{EFGHI}
ZP 753 ex	3.245 ^{DEFG}	0.310 ^{BC}	63.20 EF	550.8 ^{AB}
ZP 768	3.755 ^{A}	0.245 ^{CDEFG}	60.11 FGH	540.8 ^{ABC}
ZP 801 ex	3.425 ^{CD}	0.155 ^{IJ}	69.15 C	452.2 ^{EFGHI}
ZP 805	2.990 ^{G}	0.120 ^{J}	69.37 BC	557.3 ^{A}
ZP 808	2.695 ^{H}	0.180 ^{GHIJ}	60.11 FGH	551.5 ^{AB}
ZP 836	2.70 ^{H}	0.205 ^{EFGHI}	58.63 GH	483.0 ^{CDEFG}
ZP 846 ex	2.640 ^{H}	0.270 ^{CDE}	54.33 I	493.7 ^{BCDEF}
Average	3.26	0.24	64.45	485.35
LSD 0.05	0.281	0.066	3.44	58.6

A-L Means followed by the same letter within the same columns are not significantly different ($p < 0.05$)

Correlations were calculated to identify trait relationships that should be considered during the process of improving germplasm, as well as to compare our results with previously reported correlations to determine if similar relationships exist. The significant and positive correlation between total protein and oil content was observed (Table 3). This is in agreement, with findings of SONG and CHEN (2004), FABIJANAC *et al* (2006) and DADLEY *et al*. (2007). ALDA *et al* (2011) found positive correlation between protein and oil content of eight maize hybrids of various maturity groups. ABOU DEIF *et al.* (2012) found negative correlation between protein and oil contents in majority of studied maize inbred lines. Three inbred lines out of 14 as well as 4 crosses out of 15 had high protein and oil content. The negative significant correlation between total protein and starch content was obtained as it was expected (CLARK *et al*., 2006; DUDLEY *et al.,* 2007; HARRELSON *et al* 2008, ZHANG *et al.,* 2008, IDIKUT *et al*. 2009). COOK *et al* (2012) evaluated nested association mapping population and the 282 IL association panel for starch, protein and oil content. In both the NAM population and AP significant positive correlation were detected between protein and oil content and highly significant negative correlation between starch and both protein and oil.

Table 3. Correlation between main grain constituents of 20 ZP maize hybrids

	Total Proteins	Oil	Starch	P_i	Phytate	Sol. protein	Phenolics	Mass on 1000 grains
Total Proteins	1							
Oil	0,60748*	1						
Starch	-0,75569*	-0,51140*	1					
P_i	0,26488	0,14395	-0,36502*	1				
Phytate	0,28378	0,01450	-0,27113	0,09196	1			
Sol. protein	0,00169	0,07415	-0,22561	-0,24074	0,22811	1		
Phenolics	-0,46096*	-0,42075*	0,42132*	0,02688	-0,02088	-0,07940	1	
Mass on 1000 grains	0,11382	-0,06011	-0,41322*	-0,06761	0,36872*	0,37342*	-0,49650*	1

* $p<0.05$

A significant negative correlation of oil with starch was determined, as well as between oil and phenolics, as it was expected. It could be also assumed that the relative high oil content could be expected at hybrids with high total protein and low starch content and *vice versa*, in hybrids with low protein and high starch content (Fig. 1C).

Irrespective to fact that there was no significant correlation between total protein content and mass of 1.000 grains, it is important to underline that seeds with highest mass have total protein content in range 9-10% and starch content in range 80.5-82% (Fig. 1A), what could serve as basis for further breeding programs which include high total protein and/or oil programs (SALEEM *et al.,* 2008; ROSULJ *et al.*, 2002). The differences in oil content among hybrids are usually associated with differences in the proportion of kernel constituted by the embryo. The hybrids with the highest proportion of embryo in kernels had the highest oil content

(URIBELARREA *et al.*, 2004). This may be supported by a negative correlation between 1000 kernal weight and oil content in our research also reported by FABIJANAC *et al.* (2006).

As in many previous reports (LORENZ *et al.*, 2007, 2008; DRINIC *et al.*, 2009), phytate content in maize grain was positively correlated with proteins (r=0.28). In our study, mentioned correlation was not significant, while the correlation between phytate and mass of 1.000 grains was positive and significant. A negative correlation occurred between phytate and starch (r=-0.27). These findings is in agreement with findings of HARLESON *et al.* (2008), IDIKUT *et al.* (2009), LORENZ *et al.* (2008), who found negative relationship between starch and phytate. This result reflects the seed deposition pattern of phytate, where approximately 90% of phytate is found in the germ and only trace amounts are in the endosperm. In Figure 1D results of dealings between phytate, total protein and starch content emphasize that the highest phytate content was found in hybrids with total protein content in range 9.5 – 10.5%, as well as starch content in range 79.5 – 80.5%. Hybrids that have the low phytate concentration have also the highest starch content, but lower protein content than average. Similar to phytate, the correlation between starch and P_i was significantly negative (r=-0.36). The highest P_i content was also observed in hybrids with higher contents of proteins, as well as hybrids with relative high starch content and low protein content (Fig. 1E), indicating that breeding course for low phytate genotypes (CAST, 2002; RABOY *et al*., 1997) isn't the only way for increasing of P_i content, as digestible P form.

Additionally, the significant negative correlation was observed between total protein content and phenolics and in combination with seed mass emphasizing that seeds with lower mass had lower phytate content, as well as higher content of phenolics (> 500 µg g^{-1}, Fig. 1B).

The observed results could indicate positive traits for breeding programs because of well-known formation of indigestible protein-phenolics and protein-phytate complexes, what is the major limiting factor to protein utilization. Moreover, the significant positive correlation was obtained between starch and phenolics, as well as, the significant negative correlation between total proteins and phenolics, what could stress that relative high content of phenolics could be expected in hybrids with high content of total proteins and low starch content and in some degree in hybrids with high starch content and low protein content (Fig. 1E).

In conclusion, obtained values for protein, oil and starch are typically for maize genotypes that were not specifically selected for those traits. It s important to underline that hybrids from FAO 500 had the highest protein content, while the hybrids from FAO 800 have the highest starch content and the lowest content of total protein and phytate, indicating different endosperm constitution and potential usage. Moreover, the seeds with highest mass have total protein content in range 9-10% and starch content in range 80.5- 82% with lower values of phytate content and higher values of phenolics, what could serve as basis for further breeding programs, which include hybrids low phytate with possible high antioxidative properties, based on phenolics.

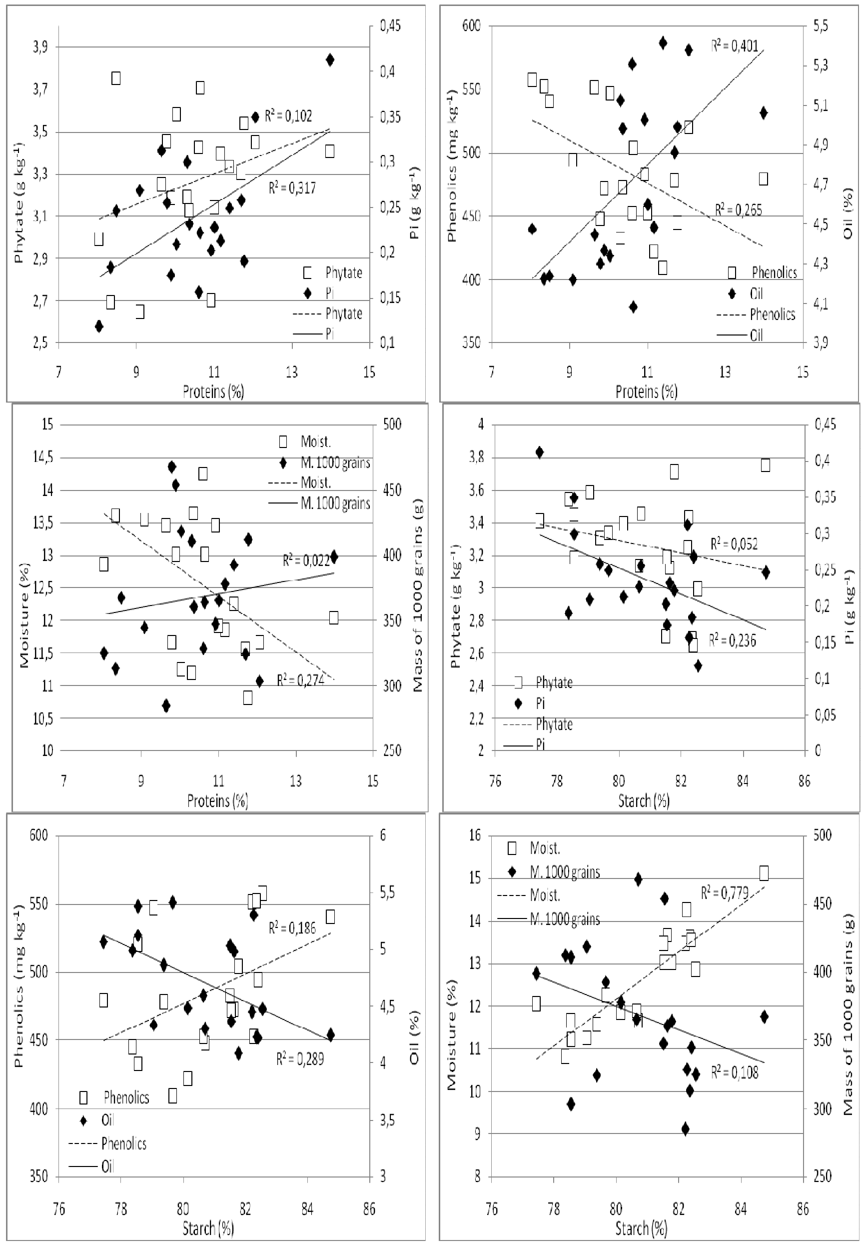

Figure 1. Relations between: Crude protein vs. phytate and P_i (A); Crude protein vs. phenolics and oil (B); Crude protein vs. mass of 1,000 grains and moisture (C); Starch vs. phytate and P_i (D); Starch vs. phenolics and oil (E); Starch vs. mass of 1,000 grains and moisture (F)

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PROCENA NUTRITIVNOG KVALITETA HIBRIDA KUKURUZA NA OSNOVU HEMIJSKE KOMPOZICIJE ZRNA

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U ovom radu je analizirana hemijska kompozicija zrna 20 hibrida kukuruza različitih FAO grupa zrenja sa ciljem da se definiše njihova nutritivna vrednost na osnovu osnovnih biohemijskih komponenti zrna: skroba, proteina, ulja, fitata neorganskog fosfora, fenola i mase 1000 zrna. Eksperiment je postavljen u u blok dizajnu (RCBD) na eksperimentalnim poljima Instituta za kukuruz u Zemun Polju tokom sezone 2009. Prosečne vrednosti proteina, ulja i skroba analiziranih hibrida kukuruza iznosile su 10.5%, 4.7% and 80.8%, respektivno. Hibridi FAO grupe zrenja 600 imali su najveću masu 1000 zrna (u proseku 419.5 g). Uočena je varijabilnost u sadržaju fitina kod analiziranih hibrida i ona je iznosila od 2.64 do 3.75, sa prosečnom vrednošću od 3.44 g kg^{-1}. Koncentracija neorganskog fosfor (Pi) se kretala od 0.120 µg g^{-1} (ZP 805) do 0.415 µg g^{-1} (ZP 588ex), sa prosekom od 0.24 µg g^{-1}. Sadržaj solubilnih proteina je varirao od 54.33 do 78.74 mg g^{-1}. Visoko pozitivna korelacija uočena je između sadržaja protein i ulja u zrnu, kao i između skroba i fenola. Fitat je bio u pozitivnoj korelaciji sa proteinima, ali ova korelacija nije bila statistički značajna, dok je korelacija između fitata i mase 1000 zrna pozitivna i signifikantna. Negativna korelacija je uočena između fitata i skroba.

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